

Abstract

Ubiquitin is a post-translational modifier regulating a plethora of cellular pathways. Deubiquitinating enzymes (DUBs) are essential within the ubiquitin system by maturing ubiquitin, which is expressed in form of fusion proteins, and regulating the ubiquitin signalling pathways by trimming of ubiquitin chains or removing them from substrates. In humans, 99 DUBs, divided into six families, were identified. This study focused on the characterization of the novel DUB ZUFSP, thereby giving rise to a new, seventh family of DUBs.

Within this study, it could be identified that ZUFSP is a specific DUB, which cleaves selectively K63-linked ubiquitin chains with a minimal length of 3 moieties in an endo-cleavage mode. Determination of the ZUFSP/Ub-complex structure provided insights into the molecular mechanism of ZUFSP, clarifying how ZUFSP gains its specificity for ubiquitin. Furthermore, the structural characterization revealed a novel ubiquitin-binding domain (UBD) named zUBD as well as a region named "α2/3 protrusion", which serve as the S1 and S1' site of ZUFSP. Together, these two regions dictate the selectivity for K63-linked chains. In addition, this study focused on the structural comparison of ZUFSP to other proteases, including members of the ATG4 and UFSF families, which were previously reported to exhibit a high sequence conservation with ZUFSP. In doing so, ZUFSP was established as the founding member of a new protease family belonging to the CA clan, which is distinct from the ATG4 and UFSF families.

ZUFSP is the sole member of this newly identified protease family in humans. Homologues from different species were analysed, revealing a highly diverse architecture of N-terminal UBDs. Comparative biochemical analysis of homologues comprising different architectures revealed a separation of the ZUFSP family in two subfamilies. On the one hand, insect ZUFSP has a similar UBD architecture as the human form and shares the preference for long, K63-linked ubiquitin chains. On the other hand, ZUFSP homologs from the yeast *S. pombe* and the plant *A. thaliana* have no or just one UBD and preferentially cleave K48-linked ubiquitin chains without selectivity for long chains. The structures of the insect and yeast homologues were solved and highlighted molecular differences in the ubiquitin-binding mode between both subgroups. Furthermore, structural comparisons allowed the identification of a putative oxyanion hole for the DUBs displaying K63-linkage selectivity, which allows to boost the activity of human ZUFSP.

Based on the thorough biochemical characterization of ZUFSP, a biological function for this novel DUB family can be proposed. In this regard, a role of ZUFSP in the homologous repair pathway after DNA double strand break (DSB) can be suggested, which is in line with the results of a previous study. First, the identified selectivity of ZUFSP for K63-linked chains supports its role in DSB repair, since these pathways are highly regulated by this particular form of ubiquitination. In addition, this study confirms the interaction of ZUFSP with the RPA complex as well as identifies novel interaction partners, such as USP7, USP11 and UBR5, which execute important functions in the regulation of DSB repair as well.

In sum, this study reveals a highly selective, novel family of deubiquitinases with the human member ZUFSP, displaying a regulatory function in the DSB repair pathway.